# THE CONTRIBUTION OF ONE COMPONENT OF A CONTROL SYSTEM TO VERSATILITY OF GENE EXPRESSION

*Barbara McClintock*

Previous reports and publications have presented evidence of the extraordinary versatility of the *Spm* (Suppressor-mutator) system in regulating gene action during development of the maize plant. The most revealing information comes from studies of those genes whose function is required for production of anthocyanin pigment, because this pigment can be produced in most parts of the plant and also because it is not required for cell viability. The pigment appears in many strains of maize, and in any one plant its distribution to the various parts is genetically controlled. The controls regulate whether or not pigment will be produced in any particular tissue, its intensity if produced, and the patterns of distribution within a tissue should the pigment not be uniformly distributed. Genetic control of distribution and intensity was recognized in early studies with maize, conducted before those that revealed the presence of regulatory components asso-

ciated with structural genes. Alleles of individual loci were identified, each of which can specify distinctive patterns of pigment distribution and intensity, and these alleles were isolated in maize derived from various sources. Phenotypic expressions attributable to the yet unknown control systems associated with these alleles resemble those resulting from some "states" of a locus under the control of a known regulatory system. In other words, alleles isolated from natural populations and those selectively isolated during investigations of known control systems may express similar properties.

Studies in recent years by many investigators have emphasized the complexities of operation of regulatory systems that function during development of eukaryotic organisms. Some of these systems may regulate gene action in much the same manner as the *Spm* system. That system is able to control the action of a number of different genes, either in similar or in different ways and at similar or different stages of development. Its versatility is impressive and instructive. Such systems may function not only to direct differentiation within individuals of a species but also to provide some of the genetic diversity responsible for the origin of new forms. In this event, new origins should be related more closely to the operation of regulatory systems than to alterations in the composition of the structural genes themselves. The system could redirect the time during development when particular genes will act, the cells of a tissue within which they can act, the quantity of their products in any one cell, and the components with which the products may be associated.

It is now well recognized that the genomes of most organisms have many of the same basic operational tools: the same kinds of enzymes, the same cellular structures with the same functions, and the same overall gene products required to build and maintain these structures. The differences between organisms, therefore, must reflect to a large extent differences in regulation of their genomes. This view leads to the conclusion that most genomes are potentially capable of constructing many distinctively different forms, and in some organisms they do just that. Witness the caterpillar and the moth: they share the same genome. Polymorphism associated with mimicry within a species also reflects such sharing. Certainly some examples of mimicry in plants and animals illustrate the extraordinary range of genome versatility. Indeed, the very fact that one genome within a eukaryotic individual can give rise to so many different organs, and also to so many distinctive types of cells, is implicit evidence of the potential range of expression of a single genome.

The operation of just one regulatory system in maize can provide a wide range of control of gene expression. The action of only two heritable units, or elements, is required to accomplish these diverse expressions. This fact suggests that the basic mechanisms responsible for such control may not be complicated in themselves. In many instances a single event occurring to an element alters the time, the type, and the pattern of gene action. Yet the effect of some of these events is reversed at particular stages in development, or the element may undergo a sequence of subsequent events, each of which conditions gene action in a specific manner.

It is reasonable to suppose that control systems resembling those explored in maize also operate in other eukaryotic organisms. With this in mind, the present report will review and illustrate one aspect of the operation of a system in maize. It is based on an extended series of studies, recently completed, of changes in the action of one component of a control system; and it will consider the responses given to these changes by only one "state" of a gene locus. This is the class II state of $a_2^{m-1}$, initially reported in *Year Book 57* and *Year Book 58*. It is a very useful state for examination of

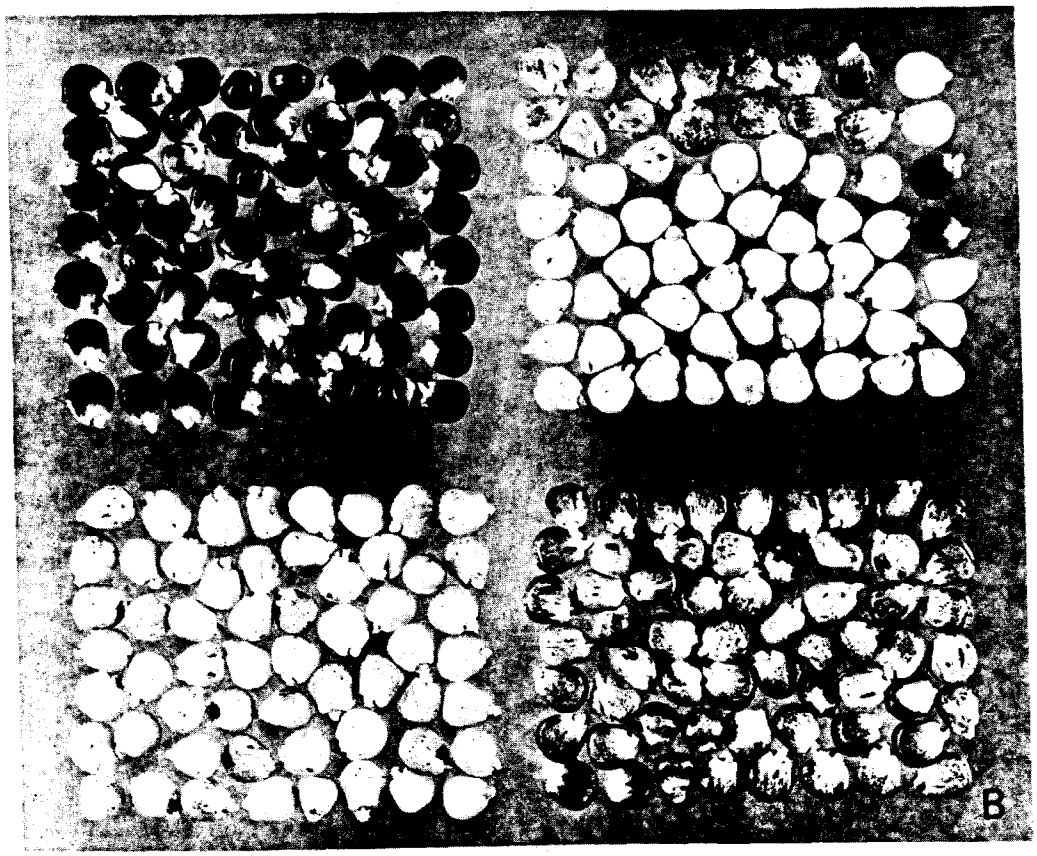
types of activity, and changes in activity, relating to the suppressor or inhibitor component of *Spm* (component-1, discussed in *Year Book 64*). When this component is active, gene expression at the $a_2^{m-1}$ locus ($A_2$, anthocyanin) is inhibited; when it is inactive, gene expression is intense, resembling that induced in plant and kernel by the standard $A_2$ locus. It is important to emphasize that this state of $a_2^{m-1}$ does not respond to the "mutator" component of *Spm* (component-2) as do so many other states. No *heritable* alterations have been noted that would modify its subsequent expression either in the presence or in the absence of an active *Spm* element. Consequently, within a plant or kernel, the distinctive patterns of pigmentation, and the modifications of pattern that may be clonally exhibited, reflect the action of the *Spm* element and the changes in action that it undergoes. The responses of the class II state of $a_2^{m-1}$ are direct and uncomplicated, yet they are capable of providing many different phenotypes. We shall consider these phenotypes and the changes in *Spm* action that are responsible for their appearance.

To orient the discussion, we may observe the phenotypes of kernels on the segment of ear shown in Fig. 2A. The ear was produced by a plant that was homozygous for the standard recessive allele of $A_2$ in chromosome 5, designated $a_2$. Plants and kernels homozygous for $a_2$ do not produce anthocyanin pigment, and this allele does not respond to *Spm*; no known change occurs at the $a_2$ locus in the presence of an active *Spm* element. In addition, the plant was homozygous for $wx^{m-8}$ in chromosome 9, an allele of the *Wx* locus. (*Wx*, amylose starch in cells of the gametophyte and the endosperm; *wx*, waxy, standard recessive, no amylose starch produced in these tissues.) The action of $wx^{m-8}$ is under the control of the *Spm* system. When an *Spm* element with active component-1 and component-2 is present in plant or kernel, the controlling element at the locus of $wx^{m-8}$ can respond to it by undergoing a somatically occurring, heritable modification that releases inhibition of gene action. The gene is then potentially active in descendants of cells in which such an event occurs, but can be expressed only in the cells of the gametophyte and in those of the endosperm. When such events occur during development of the endosperm, the clones of descendent cells are readily recognized—sometimes visually, if the clones are large, because of altered light reflection, or quite accurately by means of the blue coloration shown by the amylose starch upon exposure to an I-KI solution.

The ear-producing plant had no active *Spm*, and thus no changes occurred at the locus of $wx^{m-8}$ during the period when the ear was being formed. The kernels on this ear, however, were produced after the silks received pollen from a plant having the constitution $a_2^{m-1}/a_2$; $wx/wx$ and also one *Spm* not linked with either $a_2^{m-1}$ or $a_2$. When initially introduced into the primary endosperm nucleus, both components of *Spm* must in most instances have been in an active phase. This was revealed by the ratio of kernel types on the ear and by the responses of $wx^{m-8}$ and $a_2^{m-1}$ to *Spm* during subsequent endosperm development.

Half of the kernels on the ear, shown partially in Fig. 2A, should have received the $a_2$ allele from the pollen parent and therefore should be homozygous for this allele. Their aleurone layer should be colorless. Half of these, in turn, could have received (and did in fact receive) the active *Spm* from the pollen parent. Such kernels are recognized by the presence of clones of cells containing amylose starch, initiated by responses of $wx^{m-8}$ to component-2 of the introduced *Spm* element. The other half of the kernels on the ear should have received $a_2^{m-1}$ from the pollen parent. Those lacking an active *Spm* should have the aleurone layer uniformly and deeply pigmented. Those that received active *Spm* should have pigment production suppressed in this

A



B

layer except when changes in *Spm* activity, occurring in some cells during endosperm development, allowed pigment to be produced. Clones derived from such cells, terminating in the aleurone layer, are easily recognized as pigmented spots.

In the ear section shown in the photograph, each kernel in five adjacent rows may readily be placed in one of three classes according to pigment expression. Twenty-nine kernels are totally colorless, fourteen are uniformly and deeply pigmented, and fourteen have many specks and some spots of deep pigment in a colorless background. The kernels in the latter two classes received $a_2^{m-1}$ from the pollen parent, and the spotted kernels are the ones that received both $a_2^{m-1}$ and an active *Spm*. These constitutions are verified by the fact that none of the totally pigmented kernels has any *Wx* clones, whereas all the spotted kernels have such clones. The observed ratio of kernel phenotypes is that expected from the known constitutions of the two parents. All the other kernels on the ear—with the exception of several that commenced development with an inactive *Spm* and whose phenotype will be considered later—have phenotypes similar to those shown in the photograph, and the ratio also is the same.

It may be noted that the kernels receiving $a_2^{m-1}$ and an active *Spm* have similar patterns of pigment distribution in the aleurone layer. Patterns of this type are distinguished first by closely spaced, small spots in a colorless background, and second by the presence in most kernels of large pigmented areas and of other well-defined areas containing relatively few, widely spaced specks of pigment. In many of the kernels, these two contrasting areas are located adjacent to each other. It is probable that many of them arose from an early-occurring transposition of *Spm* that resulted in its loss from one cell and inclusion in the sister cell. A large, fully pigmented area in the aleurone layer would be formed by descendants of the cell that had lost *Spm*, and in the underlying starch-bearing cells no subclones having modified action of $wx^{m-8}$ should be found. Cells of the adjacent area would have descended from the one that received the transposed *Spm* and thus contained an extra *Spm* element. It will be shown in the following section that areas of the aleurone layer derived from such cells are expected to have the observed pigmentation patterns. It should be stressed, however, that events other than transposition of *Spm* are known to give rise to uniform deep pigmentation, or a lightly speckled pattern of pigmentation, in the aleurone layer. These will be considered later.

### *Relation of Dose of* Spm *to Pattern of Pigmentation with the Class II State of* $a_2^{m-1}$

In plants and kernels having the class II state of $a_2^{m-1}$ and an identifiable *Spm* element or elements, various distinctive patterns of pigment distribution appear. Because pigment will be produced in a cell only when there is no activity of component-1 of *Spm*, these patterns must reflect events that control the expression of that component in different cells during development of plant or kernel. Two

Fig. 2. A. Kernels in a segment of an ear of a plant (8720-8) that was homozygous for $a_2$ and for $wx^{m-8}$ and had no detectable *Spm*, produced when silks of this ear received pollen from a plant (8734D-3) having the constitution $a_2^{m-1}$ (class II state)/$a_2$; $wx/wx$, and one *Spm* with active components-1 and -2. The totally colorless kernels are homozygous for the $a_2$ allele. The kernels with pigment in the aleurone layer received $a_2^{m-1}$ from the pollen parent. Those with spots and specks of pigment in a colorless background also received an active *Spm* element from this parent. B. All the pigmented kernels that appeared on a self-pollinated ear of a plant (8735E-5) having the constitution $a_2^{m-1}$ (class II state)/$a_2$; + *Wx*/active-*Spm wx*. Kernels in the upper right quadrant are *wx* in phenotype; those in the remaining three quadrants are *Wx*. The fully pigmented kernels have no active *Spm*. The heavily spotted kernels exhibit the "1 *Spm*" pattern, whereas those with some small spots or only a few specks of pigment have a "2 *Spm*" or "3 *Spm*" pattern.

main types of event are known to accomplish this. One is transposition of an active *Spm*, as outlined in the previous section. This event is controlled by component-2 of *Spm*, but will not occur unless component-1 also is active. Component-2 is the "mutator" component of the *Spm* system, inducing heritable modifications of the elements of the system that are incorporated at various gene loci, as well as transposition of these elements and also transposition of the regulator element *Spm* itself. As stated earlier, the class II state of $a_2^{m-1}$ is unique in that it does not respond to component-2. This component undergoes quite specific types of modification, recognized by changes in the time or frequency of occurrence of transpositions. Some of the patterns produced by $a_2^{m-1}$ reflect such changes.

The second kind of event occurring to the *Spm* element and affecting patterns of pigmentation involves change in phase of activity of its component-1, from active to inactive and back to active, without an associated change in location. The time and frequency of occurrence of the phase changes reside, to a large extent, in the *Spm* element itself, and their control appears to be reset with each change. Thus, the pigmentation patterns observed in plants and kernels having the class II state of $a_2^{m-1}$ relate basically to conditions that regulate the occurrence of these two distinctly different types of events involving *Spm*—its transposition, and change in phase of activity of its component-1. If more than one *Spm* element is present, the patterns reflect the events affecting each. Therefore the number of *Spm* elements initially introduced into a plant or into the endosperm of a kernel, and the changes in number that may subsequently occur, are effective means of modulating pigmentation patterns, as is illustrated by the phenotypes of the kernels shown in Fig. 2B.

These kernels have either uniform pigmentation or pigmented spots (many or few) in a colorless background. They were produced on the self-pollinated ear of a plant having the constitution $a_2^{m-1}/a_2$; + *Wx*/active-*Spm wx*. Only one identifiable *Spm* element was present in the plant, and it was closely linked with the *wx* allele. In addition to the 245 kernels shown in the photograph, there were 106 totally colorless kernels, of which 85 were *Wx* and 21 *wx*. In the photograph, the kernels are arranged first with regard to the *Wx* and *wx* phenotypes, and then according to pigment expression in the aleurone layer within each of the two classes. All 64 kernels in the quadrant at upper right have the *wx* phenotype. The kernels in the other three quadrants are *Wx*, and 64 of them are uniformly pigmented (upper left). The remaining 117 *Wx* kernels have pigmented spots in a colorless background, but the patterns obviously are not all alike. The kernels in the lower right quadrant have many spots of pigment, some of them large. Most of the kernels in the group at lower left have smaller spots, and a few have only several very small specks of pigment.

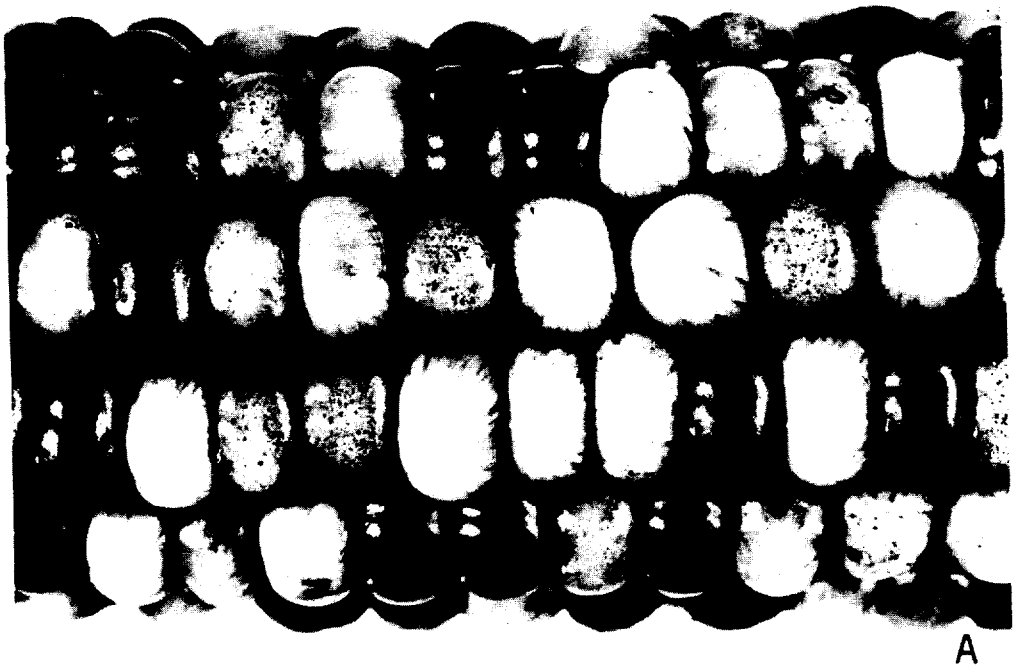
The assumed ratio of *Spm* constitutions initially present in the endosperm of the kernels in the photograph would be 1 with no *Spm* : 1 with one *Spm* : 1 with two *Spm* : 1 with three *Spm*. This ratio relates to the three sets of chromosomes introduced into the primary endosperm nucleus, two sets from the female gametophyte and one set from the male gametophyte (pollen grain). The anticipated ratio with regard to the *Wx* alleles is 1 *Wx* /*Wx*/*Wx*: 1 *Wx*/*Wx*/*wx*: 1 *Wx*/*wx*/*wx* : 1 *wx*/*wx*/*wx*. The relatively close linkage of *Spm* with the *wx* allele in the parent plant should distribute the *Spm* element in close accordance with this ratio. The kernels on the ear should reveal this relationship, provided that no early-occurring transpositions or inactivations of *Spm* occurred in the plant to effectively alter expected gametic ratios with respect to *Spm* constitution or activity. Such events did not occur to any distorting extent, as the ratio of kernel phenotypes indicates. Of the 66 kernels

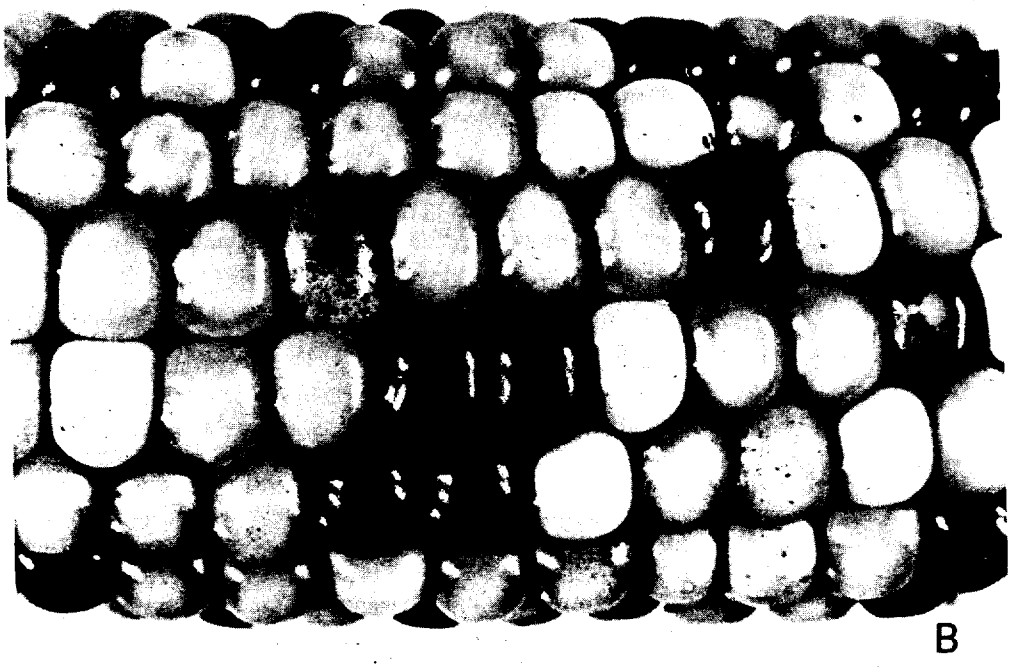
that are uniformly pigmented (no evidence of *Spm*), 64 are *Wx* and 2 are *wx* (the two kernels at the right in the *wx* quadrant). The 60 kernels in the lower right quadrant have a "1 *Spm*" pattern of pigmentation. Most of those in the lower left quadrant have a "2 *Spm*" pattern. The several kernels with only a few tiny pigmented specks exhibit a "3 *Spm*" pattern. The majority of kernels in the *wx* quadrant (upper right) exhibit this "3 *Spm*" pattern. Nine kernels, however, placed in the upper part of the quadrant, have a typical "1 *Spm*" pattern, and seven others adjacent to them show the "2 *Spm*" pattern.

This classification for *Spm* constitutions was confirmed by several kinds of test. In one test, pollen of the plant was used not only for self-pollination but also in a cross similar to that which produced the kernels shown in Fig. 2A. Only one *Spm* is expected to be present in most of the pollen grains that carry it. Therefore most of the spotted kernels should exhibit the "1 *Spm*" pattern, and in tests of a number of plants this expectation was fulfilled. Another test utilized a second ear of the plant. Its silks received pollen from a plant that was homozygous for the class II state of $a_2^{m-1}$ and also for *wx* but did not have an identifiable *Spm* element. From such a cross, all or nearly all kernels that received an active *Spm* should show the "2 *Spm*" pattern, and linkage of this *Spm* with the *wx* phenotype should be sharply expressed. Again, the expected relationships were observed. (Occasionally on such ears, one or several kernels appear that exhibit the "1 *Spm*" pattern or a high-dose *Spm* pattern. Most of these aberrant phenotypes are related to transpositions of *Spm* that occur either during development of the female gametophyte, allowing entrance of only one *Spm* into the primary endosperm nucleus, or at an earlier stage, providing the female gametophyte with extra *Spm* elements.) A third and extensive series of tests was conducted with plants derived from kernels exhibiting different phenotypes, taken from ears produced by each of the described crosses. Again, each plant was examined for *Spm* number, location, and activity. From the results of these multiple tests it was possible to interpret the origins of the different pigmentation patterns, and to know when to relate some of them to dose of *Spm*.

A second illustration of the relation of pattern to dose of *Spm* is given by the kernels on the segment of ear shown in Fig. 3A. This was a self-pollinated ear of a plant having the constitution $a_2^{m-1}/a_2^{m-1}$; + *Wx*/active-*Spm* *wx*. Pigment was produced in two distinctly different layers of the kernels, the aleurone layer of the endosperm and the overlying pericarp layer. The pericarp layer is derived from cells of the ovary and thus has the same genotype as other tissues in the plant. It is a maternal layer. In order that pigment may be formed in various parts of a plant, genes associated with anthocyanin production other than that at the $a_2^{m-1}$ locus must be capable of functioning in these parts. In some studies it was necessary to incorporate effective alleles of such genes into the genome of a plant in order to examine pigment production in its various parts that could be attributed solely to activity of the gene at the $a_2^{m-1}$ locus. The plant producing the ear shown in Fig. 3A was so constituted.

The streaked pigmentation patterns in the pericarp layer are best seen in the waxy kernels having a nearly colorless aleurone layer, although similar patterns are present in all the other kernels as well. The streaks run from the base of the kernel toward the crown, narrowing in width as the crown is approached, and are relatively uniform in size and distribution. The pigmented spots in the aleurone layer of the highly light-reflective *Wx* kernels having a "1 *Spm*" pattern also appear to be relatively uniform in size and distribution. There are no large spots in any of these kernels. The events affecting *Spm* that were re-


A


B

sponsible for these patterns occurred in both plant and endosperm tissues at a relatively late developmental stage. This late timing is also reflected in the *wx* class of kernels. Most of them commenced development with three *Spm* elements in the primary endosperm nucleus, and most of them have only a few specks of pigment in the aleurone layer. The patterns produced by *Spm* in this plant differ from those appearing among the kernels shown in Fig. 2A and B, and the distinctions are related to differences in control of the timing of some types of events affecting the *Spm* element.

A third illustration of the connection between pigmentation pattern and dose of *Spm* is shown in Fig. 3B. The kernels were produced on the self-pollinated ear of a plant with the constitution $a_2^{m-1}$/$a_2^{m-1}$; inactive-*Spm* *Wx*/active-*Spm* *wx*. When an inactive *Spm* is present in the same nucleus with an active *Spm*, the inactive element contributes to the phenotypic expression as though it were in an active phase. When it is removed from this association by meiotic segregation, the activity also is removed, and the phenotypic response of the class II state of $a_2^{m-1}$, or of any gene locus under the control of the *Spm* system, is the same as though no detectable *Spm* were present. These different expressions appear among the kernels on the ear segment shown in the photograph. The deeply and uniformly pigmented kernels are *Wx*. Several other kernels have deep pigment over most of the aleurone layer but also some colorless areas with ill-defined borders. These also are *Wx*. Their *Spm* was in the inactive phase initially but underwent change to the active phase in some cells during endosperm development. All other kernels on this segment of the ear commenced endosperm development with at least one active *Spm*. The ratio of the phenotypes and their distribution among the *Wx* and *wx* classes make it evident that most of the *Wx* kernels must have received the *Spm* that was linked to this *Wx* allele, and also that component-1 of this *Spm* was in the inactive phase. Yet, among the kernels with pigmented spots or specks, there is little difference in pattern between those that are *Wx* and those that are *wx*. Both exhibit the "high dose" *Spm* pattern. There are a few kernels on this ear, however, that show what was previously described as a "1 *Spm*" pattern, and one waxy kernel with this pattern is conspicuous in the photograph. As indicated earlier, a few kernels having this phenotype could be anticipated.

### *Distinctive Phenotypes Associated with Activation of an Inactive* Spm

The contribution of an inactive *Spm* to pattern of pigmentation, as illustrated in Fig. 3B, provides a means of detecting the presence of inactive *Spm* elements in plants that otherwise show no evidence of *Spm*. Pollen from a plant carrying a single *Spm* element in the active phase may be placed on the silks of an ear of a plant to be tested. From the types of pigmentation patterns appearing among

Fig. 3. A. Segment of an ear showing the types of kernels produced by self-pollination of a plant (8735E-1) that was homozygous for the class II state of $a_2^{m-1}$ in chromosome 5 and had the alleles + *Wx*/active-*Spm* *wx* in chromosome 9. The shiny kernels are *Wx* whereas the dull, waxy-looking kernels are *wx*. The genetic constitution of the plant allowed pigment to be produced in the pericarp layer, which is maternal in origin. The streaks running from the base toward the crown are in this layer. Those kernels that are uniformly dark or have a spotted pattern contain pigment also in the aleurone layer of the endosperm. B. Segment of an ear produced by self-pollination of a plant (8731A-1) that was homozygous for the class II state of $a_2^{m-1}$ and had an inactive *Spm* linked with *Wx* in one chromosome 9 and an active *Spm* linked with *wx* in the homologue. The dark-colored kernels received only the inactive *Spm*. The nearly colorless kernels received at least one active *Spm*. These kernels exhibit a "high-dose" *Spm* pattern. Note the contribution of the inactive *Spm* to dose expression (see text). One kernel with a *wx* phenotype has a "1 *Spm*" pattern of pigment distribution.

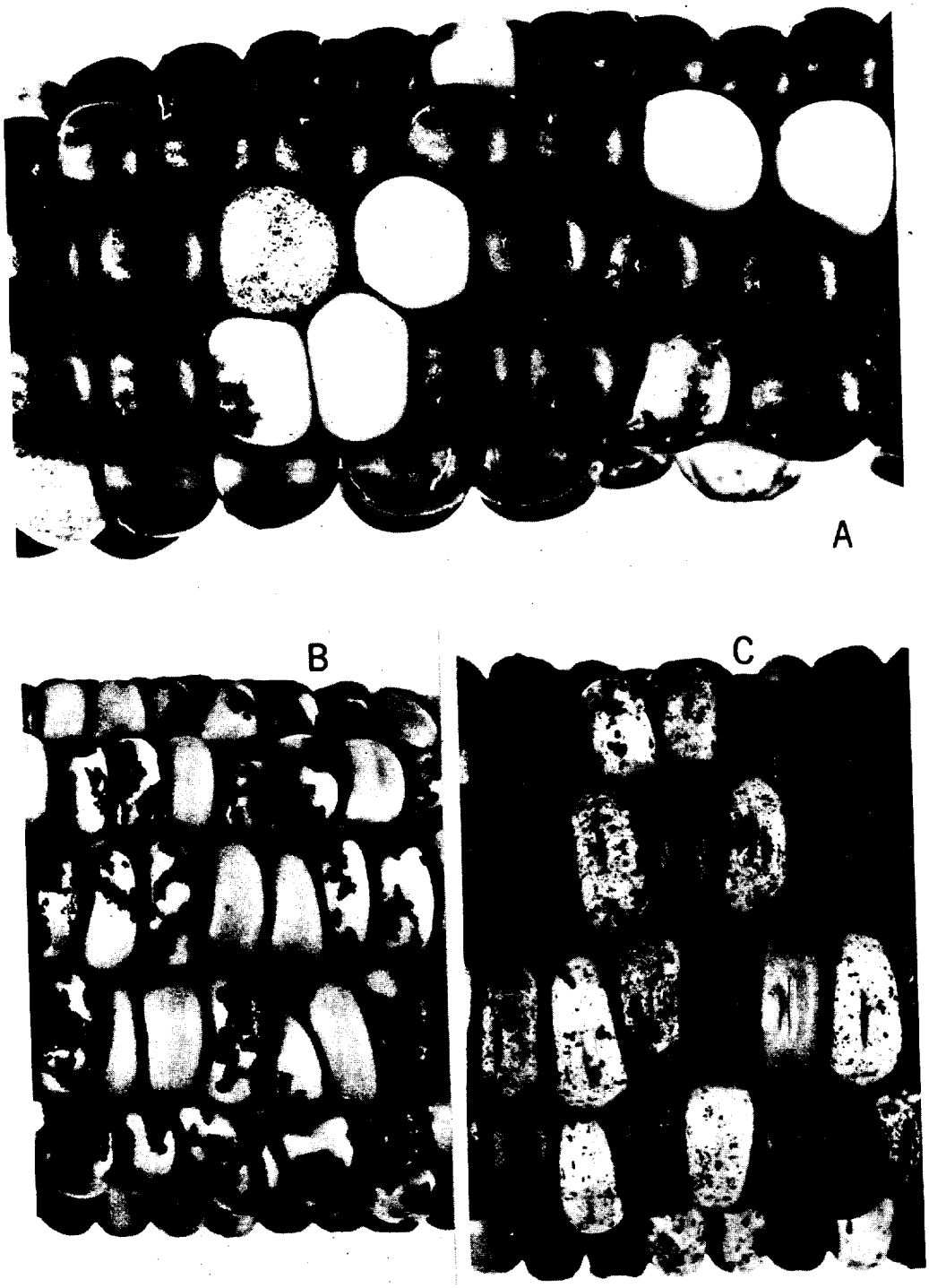
the kernels, one can learn whether or not the plant carries an *Spm* whose component-1 is inactive. Such tests made it possible to examine the duration of inactive phases of component-1 that extended, in some instances, over generations of plants. Returns to the active phase, when they occur, may be noted by the appearance of clones of cells either in the plant or in the kernel. In the kernel, such changes of phase can initiate some very distinctive pigmentation patterns, several of which are illustrated in Fig. 4A.

The kernels shown here appeared on the self-pollinated ear of a plant that was homozygous for the class II state of $a_2^{m-1}$ and had one *Spm* element undergoing changes in phase of activity during plant development. In most parts of the main stalk it was in the inactive phase, and in most of the kernels on the ear produced by this stalk it was also inactive. In some kernels, however, it entered the active phase; several of these appear in the photograph. Four of them are nearly colorless, having only a few specks of pigment. Two other kernels in the photograph have pigment confined to the crown in the region surrounding the silk attachment, and the borders of the pigmented areas appear frayed. Other kernels have large pigmented areas with frayed borders that are adjacent to colorless areas with specks of pigment; two kernels showing this phenotype may be seen at the lower right. A few kernels have very many tiny specks of pigment, uniformly distributed over the aleurone layer; two of these appear in the photograph. It was learned, in studies of plants derived from kernels with phenotypes similar to those shown here, that the endosperm in each of the kernels probably received initially an inactive *Spm*, even though several of the pigmentation patterns resemble those produced by different doses of an active *Spm* as previously illustrated. These quite distinctive patterns demonstrate still other instances of versatility of regulation of gene expression related to the action of only one component of one element of one control system.

### *An Example of Versatility of Control of Gene Expression Associated with Component-2 of* Spm

The illustrations given so far demonstrate only one aspect of the versatile

Fig. 4. A. Part of an ear produced by self-pollination of a plant (8738-15) that was homozygous for the class II state of $a_2^{m-1}$. It had an *Spm* whose component-1 was in an inactive phase in most parts of the plant. The nearly colorless kernels as well as those with different patterns of pigment distribution received an inactive *Spm*, which underwent change to active phase during development of the endosperm. Note the range of pigmentation patterns that this mechanism may produce. B. Types of pigment distribution in the aleurone layer of kernels on an ear of a plant (7896B-1) that was homozygous for $a_2$ and had two unlinked, active *Spm* elements. The silks of this ear received pollen from a tester plant that was homozygous for the original state of $a_2^{m-1}$ and had no detectable *Spm* element. The uniformly pale-pigmented kernels show the phenotype produced by this state when *Spm* is not present in the endosperm. The variegated kernels have large and small areas of deep pigment. This pattern is produced by the original state of $a_2^{m-1}$ in response to an *Spm* having an active component-1 and an early-acting component-2. The pigmented areas reflect the formation of clones of cells, each derived from a cell with a newly produced, heritable modification of the $a_2^{m-1}$ locus. C. Types of kernels on an ear of a plant (8448C) having the constitution $A_2/a_2^{m-1}$ (original state); $wx/wx$. The ear was produced by a cross with a tester plant that was homozygous for the original state of $a_2^{m-1}$ and for $wx^{m-8}$ and had no detectable *Spm*. The uniformly dark-pigmented kernels received the $A_2$ allele from the ear parent. The uniformly pale kernels received the $a_2^{m-1}$ allele and no active *Spm*. The kernels with a pattern of small pigmented spots in a colorless background received one or more *Spm* elements from the ear parent. These elements had an active component-1, and a component-2 to which the $a_2^{m-1}$ locus and also the $wx^{m-8}$ locus responded late in endosperm development. Compare this action of component-2 to that provided by the *Spm* elements in the kernels shown in B of this figure.


A
B
C

regulation of gene action by the *Spm* system. Previous reports have discussed the contribution of the states of a gene locus to variety of expression, as well as some of the modulations brought about by differences in action of component-2, the "mutator" component. Although these aspects will not be reviewed here, it should be emphasized that phenotypes resembling some of those shown here may be produced by combinations of other states of $a_2^{m-1}$ with selected isolates of *Spm*. These other states (the class I states) respond to both component-1 and component-2. The responses to component-2 result in *heritable* modifications affecting the subsequent expression of the gene at the locus. The timing of these responses is regulated not only by the state of the gene locus but also by the manner of action of component-2. (For these heritable modifications to occur, component-1 also must be in the active phase.) Component-2 undergoes changes that modify its action, and each change is the consequence of a single event. Each modification may be detected by the altered timing of responses of the various class I states of gene loci controlled by the *Spm* system. As with component-1, the duration of any one type of action of component-2 may extend over generations of plants, or, on the other hand, subsequent modifications may occur at more frequent intervals. The frequency of change—again as with component-1—seems to be under some form of control. Initially, component-2 may be totally inactive, or it may be effective at particular stages in development, ranging from early to late.

The photographs in Fig. 4B and C illustrate differences in gene expression related to differences in action of component-2 of *Spm*. The kernels shown in B developed on an ear of a plant that was homozygous for the standard recessive, $a_2$. A testcross was made to determine the *Spm* constitution of the plant. Pollen from a plant that was homozygous for the *original* state of $a_2^{m-1}$, and had no active *Spm*, was placed on the silks of this ear. The variegated kernels are the ones that received an active *Spm* from the ear parent, whereas the uniformly, lightly pigmented kernels did not. This pale phenotype characterizes the expression of the original state when there is no activity of component-1 in the endosperm cells. (It contrasts with the deep pigmentation that is produced by the class II state under similar circumstances even though the class II state was derived directly from the original state.) When component-1 is active, pigment production is inhibited. When component-2 also is active, this state of $a_2^{m-1}$ responds to it by undergoing somatically occurring heritable modifications, many of which prepare the locus to function in pigment formation. In the endosperm of the kernel, such responses in individual cells produce clones that may terminate in the aleurone layer, forming areas that are deeply pigmented. The intensity of pigmentation in such areas simulates that produced by the standard $A_2$ locus, or by the class II state when component-1 is inactive. The variegated kernels in Fig. 4B have such areas, and many of them are large—an indication that the events responsible for them occurred at the locus of $a_2^{m-1}$ early in endosperm development.

The very same state of $a_2^{m-1}$ that gave rise to the variegated kernels shown in Fig. 4B was also responsible for the variegated kernels on the ear shown in C of this figure. It is obvious that the patterns of pigment distribution in these kernels are very different from those seen in photograph B. The distinctions relate to differences in action of the *Spm* elements present in the plants that produced the two ears, and most directly, to differences in their component-2. The kernels in C appeared on an ear of a plant with the constitution $A_2/a_2^{m-1}$ (original state); $wx/wx$. This ear received pollen from a plant that was homozygous for the original state of $a_2^{m-1}$ and for $wx^{m-8}$, and had no *Spm*. In half of the kernels

that developed on the ear the aleurone layer is uniformly and very deeply pigmented. This was to be expected, as half of the kernels should have received the $A_2$ allele from the ear parent. The other kernels are homozygous for the original state of $a_2^{m-1}$. A few of them show no evidence of the presence of an *Spm* element. They are uniformly pale pigmented, as are their counterparts in Fig. 4B. The others exhibit a relatively uniform pattern of pigmented spots in a colorless background. These small spots and specks reflect heritable changes at the locus of $a_2^{m-1}$, induced by component-2 of *Spm* and occurring late in the development of the endosperm. A similar late timing of responses was registered by $wx^{m-8}$: clones of cells containing amylose starch are small.

The spotted pigmentation pattern shown in Fig. 4C resembles that of the single heavily spotted kernel in Fig. 3B. The controls of pattern are very different in these two examples, however, and the events responsible for pigment production are not the same. One pattern (Fig. 3B) is related to phases of activity of component-1 of *Spm*, with no heritable modifications occurring at the locus of $a_2^{m-1}$, whereas the other depends on heritable modifications at this locus, requiring the active phase of both components of *Spm*. Component-2 regulates the time of occurrence of these heritable modifications.

A spotted or speckled pattern may be produced by still other means. One of these employs selected states of $a_2^{m-1}$ that give a spotted pattern in conjunction with an *Spm* having the properties shown in Fig. 4B, and a very light speckled pattern with an *Spm* having the properties shown in C of this figure. In short, a spotted or speckled pattern may be produced within this control system by various states of a gene locus, whose expressions can then be modified by the different times and types of action of the components of the *Spm* element itself.

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## PERSONNEL

*Year ended June 30, 1971*

Elizabeth M. Bocskay, Chief Clerk
Elizabeth Burgi, Associate in Microbiology
Agnes C. Fisher, Secretary to Director; Editor
Alfred D. Hershey, Director
Laura J. Ingraham, Research Assistant
Barbara McClintock, Distinguished Service Member
Shraga Makover, Carnegie Institution Fellow
David H. Parma, Carnegie Institution Fellow
Jennie B. Pope, Curator of *Drosophila* Stocks
Irwin Rubenstein, Special Fellow, National Institute of General Medical Sciences; temporary staff member
Carole E. Thomason, Technical Assistant